

In the Claims

Claim 25 (currently amended) A method of operating absorbance-based chemical sensors to achieve calibration-free measurements, the method comprising the steps of:

- a) establishing wavelength accuracy to within about 2-3 nanometers;
- b) eliminating stray light at all wavelengths to about less than 0.1% incident light;
- c) preparing an analyte-selective reagent at a concentration;
- d) equilibrating the analyte-selective reagent to an analyte;
- e) taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a first wavelength (I_{λ_1}) with a reagent-based optical chemical sensor, wherein the sensor has been modified to allow the renewal of an analyte-selective reagent, wherein the first wavelength corresponds to an un-reacted form of the analyte-selective reagent, and taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a second wavelength (I_{λ_2}), wherein the second wavelength corresponds to a reacted form of the analyte-selective reagent;
- f) replacing the equilibrated analyte-selective reagent and analyte with a spectrophotometric blank solution;
- g) taking an intensity reading of the blank solution at the first wavelength ($I_{\lambda_{10}}$), and taking an intensity reading of the blank solution at the second wavelength ($I_{\lambda_{20}}$);
- h) calculating an absorbance ratio using the equation $A_R = A_{\lambda_1}/A_{\lambda_2}$, where A_R is the absorbance ratio, A_{λ_1} is absorbance at the first wavelength and A_{λ_2} is absorbance at the second wavelength and, wherein A_{λ_1} and A_{λ_2} are determined by

$$A = -\log (I_1/I_{10})$$

$$A_{\lambda} = -\log \frac{I_{\lambda}}{I_{\lambda_0}}$$

; and

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i) calculating the sensor response with the molar absorptivities (ϵ) of the reacted (a) form of the analyte-selective reagent and the un-reacted form (b) of the analyte-selective reagent using the equation

$$R = -\log \left(\frac{A_R - \epsilon_{\lambda_{1a}} / \epsilon_{\lambda_{2a}}}{\epsilon_{\lambda_{1b}} / \epsilon_{\lambda_{2a}} - A_R \epsilon_{\lambda_{2b}} / \epsilon_{\lambda_{2a}}} \right) = + pK_a - pH$$

$$R = -\log \left(\frac{A_R - \epsilon_{\lambda_{1a}} / \epsilon_{\lambda_{2a}}}{\epsilon_{\lambda_{1b}} / \epsilon_{\lambda_{2a}} - A_R \epsilon_{\lambda_{2b}} / \epsilon_{\lambda_{2a}}} \right) = + pK_a - pH$$

wherein when the analyte-selective reagent is prepared accurately and reproducibly at the concentration sensor readings between sensors are calibration-free.

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